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Abstract

Copper-contaminated soils were managed with aided phytoextraction in 31 field plots at a former wood preservation site, using a single incorporation of compost (OM) and dolomitic limestone (DL) followed by a crop rotation with tobacco and sunflower. Six amended plots, with increasing total soil Cu, and one unamended plot were selected together with a control uncontaminated plot. The mobility and phytoavailability of Cu, Zn, Cr and As were investigated after 2 and 3 years in soil samples collected in these eight plots. Total Cu, Zn, Cr and As concentrations were determined in the soil pore water (SPW) and available soil Cu and Zn fractions by DGT. The Cu, Zn, Cr and As phytoavailability was characterized by growing dwarf beans on potted soils and determining the biomass of their plant parts and their foliar ionome.

Total Cu concentrations in the SPW increased with total soil Cu. Total Cu, Zn, Cr and As concentrations in the SPW decreased in year 3 as compared to year 2, likely due to annual shoot removals by the plants and the lixiviation. Available soil Cu and Zn fractions also declined in year 3. The Cu, Zn, Cr and As phytoavailability, assessed by their concentration and mineral mass in the primary leaves of beans, was reduced in year 3.

1. Introduction

Phytoextraction is a less invasive, low-cost phytotechnology, which use the plants and their associated microorganisms to extract and translocate metal(loid)s from the soil to the harvestable plant parts. This technique aims at reducing either the total or extractable PTTE concentrations in contaminated soils to targeted levels, depending on the country legislation, within a reasonable time frame. In addition, plants used to phytoextract PTTE from contaminated soils must be tolerant to PTTE and adapted to the local soil and climate characteristics and biotic interactions (Keller et al., 2003). Phytoextraction is more economically feasible if, in addition to PTTE removal, crops produce biomass with an added value such as biofuel for the energy sector (e.g. oilseed, poplar and willow short rotation coppices), fibers, essential oils and biosourced chemicals for ecocatalysis (Schwitzguébel et al., 2002; Vassilev et al., 2004; Li et al., 2012). Phytoextraction can be applied on contaminated soils in combination with soil conditioners (so-called aided phytoextraction) to promote the biomass production (Tangahu et al., 2011). Phytoextraction using sunflower (*Helianthus annuus* L.) has several advantages, such as the plant's ability to accumulate moderate PTTE concentrations and extract PTTE such as Zn, Pb, Cd and Cu from the soil due to its high biomass production (Nehnevajova et al., 2007a, 2009; Herzig et al 2014).

The PTTE accumulation in sunflower shoots depends on several soil and plant factors, notably PTTE exposure, root uptake, root-to-shoot translocation, rooting depth and density, impacts of pests, pathogens, herbivores (Vassilev et al., 2002), soil pH, nature of the sorbents, presence and concentration of organic and inorganic ligands, including humic and fulvic acids, root exudates, microbial metabolites and nutrients (Efremova and Izosimova, 2012).

This work aimed at assessing the ability of a sunflower - tobacco crop rotation to remediate Cu-contaminated soils, with and without an initial single addition of compost (5%) and dolomitic limestone (0.2%). For Cu, Zn, Cr and As, the hypotheses tested were: (1) decreases in total dissolved concentrations in the soil pore water (SPW); (2) decreases in available concentrations in the soil; and (3) decreases in the phytoavailability for bean plants. Six amended plots with increasing total soil Cu, i.e. 163, 268, 382, 518, 753, and 1170 mg Cu/kg, and one unamended plot (832 mg Cu/kg) were selected out of 28 field plots. Total dissolved concentrations of Cu, Zn, Cr, and As were quantified in SPW sampled by Rhizon moisture probes to assess their mobility. The soil exposure intensity of Cu and Zn was determined by DGT (diffusive gradients in thin films) probes. Phytoavailability of Cu, Zn, Cr and As was characterized by cultivating dwarf beans on potted soils and analyzing the ionome of primary leaves.

2. Material and Methods

2.1. Site and Soils

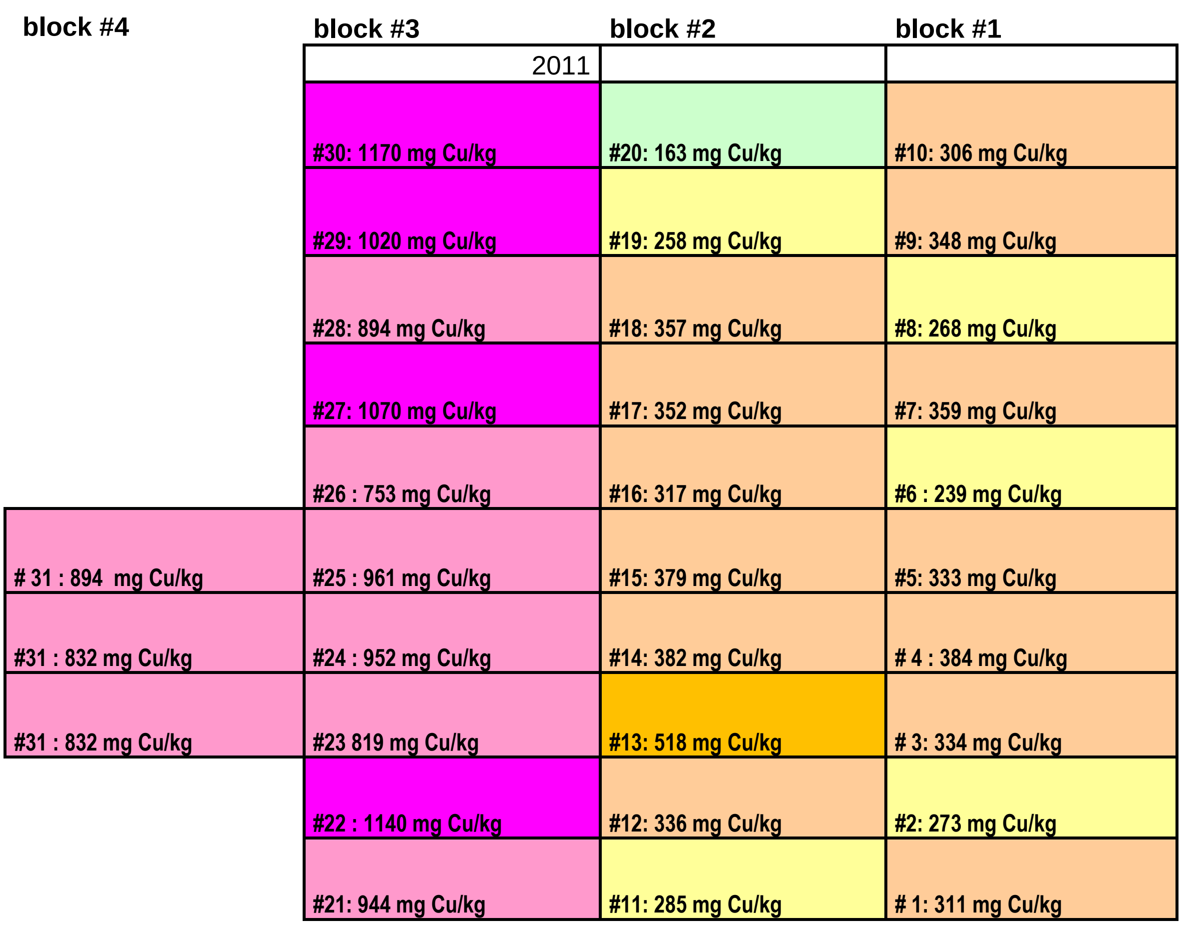
The wood preservation site (6 ha) is located in the Gironde County (44°43'N; 0°30'O), southwest France. It has been used for over a century to preserve and store timber, posts and utility poles and various Cu-based salts were successively utilized (Mench and Bes, 2009). The soil is developed on an alluvial terrace (Fluviosol) containing alluvial materials from the Garonne River combined with wind deposits (BRGM, 1978). Its texture is sandy, i.e. 85.8 % sand, 5.9 % clay, and 8.3 % silt. It contains 1.6 % of organic matter (OM), and has a C/N ratio of 17.2, with generally a low cation exchange capacity (CEC, 3.5 cmol kg^{-1}) (Bes and Mench, 2008). Copper is the major contaminant of topsoils, albeit with high spatial variation (65–2400 mg Cu kg^{-1} soil DW). Total soil As, Zn, and Cr, i.e. 10–53 mg As, 21-68 mg Zn and 20–87 mg Cr kg^{-1}, remain relatively low in the topsoils, close to background values (Mench and Bes, 2009). The site was divided into fifteen sub-sites according to past and present activities, plant communities, employees' evidence, aerial and archival photos and site history (Bes et al 2010; Kolbas et al., 2011).

The field trial, located at the P1-3 sub-site, consists in four blocks (2 m × 10 m), i.e. block #1: plots #1 to #10, block #2: plots #11 to #20, block #3: plot #21 to #30 and block #4: plot 31 (Kolbas et al., 2011). Plots #1 to #30 were amended in March 2008 with compost (5% w/w) and dolomitic limestone DL (0.2% w/w) (Kolbas et al., 2011) based on a previous pot experiment (Bes and Mench, 1998). Block #4 remained unamended and was considered as a single plot (UNT #31). Soil amendments were carefully mixed in the topsoil (0-0.25 m) with a stainless steel spade. Each amended block was divided into 10 plots (1 m × 2 m) (Fig. 1). An uncontaminated plot (1 m × 2 m) from a kitchen garden (CTRL, Gradignan, France), located at 18 km from the site, from the same alluvial terrace and with a similar soil type, was also studied. For detailed data on soil parameters in all plots see Kolbas et al. (2011).

The cropping history was sunflower in 2008 and 2009, tobacco in 2010 and sunflower in 2011 (Kolbas et al., 2011; Kolbas 2012; Mench et al., 2012). In April 2010, three soil samples (1.5 kg soil FW, 0–25 cm soil layer) were collected in six amended plots with increasing total soil Cu, i.e. 163 (#20), 268 (#8), 382 (#14), 518 (#13), 753 (#26) and 1170 (#30) mg Cu/kg, in the unamended plot (832 mg Cu/kg) (Fig.1), and in the control plots (CTRL). The three samples from each plot were mixed with a stainless steel spade and combined to produce composite soil samples (1 kg FW) which were air-dried, sieved (<2 mm, nylon mesh) and manually homogenized.

The same plots were resampled in April 2011 to investigate the phytoextraction effects on the mobility, soil exposure intensity and phytoavailability of PTTE in the soils.

Figure 1: Diagram of total Cu concentration in the four blocks (mg kg^{-1}), adapted from Kolbas et al., 2011. Colors from red to purple reflect the increase in soil Cu contamination.



2.2. Sampling and analysis of soil pore water

One kg of air-dried soil from each plot (i.e. 163, 268, 382, 518, 753, 1170, 832 and CTRL) was potted (1.3L) after sieving (2 mm). Soil pore waters (3X10 ml) were extracted from each pot by Rhizon soil moisture samplers (SMS, model MOM, Rhizosphere Research Products, Wageningen, The Netherlands). A full description of the SPW extraction procedure is given in Hattab et al. (2014).

The SPWs were stored at 4°C. A fraction of each soil pore water was acidified to 0.1 mol/L HNO_3 to measure the concentrations of Cu, Zn, Cr, and As in the samples by HR-ICP-MS (Element 2, Thermofischer). The rest of the solutions were kept for the following analyses: pH, electrical conductivity (EC), and dissolved organic carbon (DOC) which was determined by a carbon analyzer (Shimadzu© TOC 5000A). The total organic carbon (TOC) was determined in the soil by Rock-Eval pyrolysis. This instrument uses a ramped temperature pyrolysis technique whereby a small amount of soil sample (70 -100 mg) is heated in an inert atmosphere (helium or nitrogen) and combusted with air to obtain several key geochemical parameters such as the total organic carbon (TOC).

2.3. Soil exposure intensity

Bioavailable concentrations of Cu, Zn and Cr in the soils were measured by DGT devices with an active surface area of 3.14 cm^2. These probes consist of a plastic base containing three layers: the first one is a 0.45µm filter, the second is a polyacrylamide gel diffusion layer and the third one a polyacrylamide gel incorporating a Chelex-100 resin that strongly binds the labile trace metal species (Davison et al., 2000; Ernstberger et al., 2002).

The total mass of each metal (M), the flux, $F(t)$, of metal from the soil to the resin-gel, the available concentration, C_{DGT}, and the ratio, R, which indicates the extent of the depletion of soil pore water concentrations at the DGT interface are detailed in Hattab et al. (2014). Finally, the mass of each metal accumulated in the resin-gel layer was determined after extraction of the resin gel by 1 mL of HNO_3 (Suprapure, Marck Darmstadt, Germany) 5% for 24h. This solution was further diluted 10 times with HNO_3 2% and analyzed by HR-ICP-MS (Element 2, Thermo Fischer) to determine metal (Cu, Cr and Zn) concentrations.

2.4. Plant testings

Plant testing's were carried out on the soil samples collected in 2010 and 2011 (4 plants/pot), using dwarf beans and a 2-week growing period, to assess the PTTE phytoavailability. The culture and harvest conditions and the digestion of primary bean leaves (BL) were described in Hattab et al. (2014). The Cu, Cr, As and Zn concentrations in the leaf digests were determined by ICP-MS (Varian 810-MS) using standard solutions of trace elements diluted from a stock solution 1,000 ppm (±1 %/certified). Foliar element concentrations are expressed in mg kg^{-1} DW. The mineral mass of each element in the bean primary leaves was computed based on its foliar elemental concentration and the biomass (DW) of primary leaves.

2.5. Statistical analysis

Total element concentrations in the SPW, DGT concentrations, R ratios, foliar element concentrations, mineral masses of elements in the primary leaves and leaf DW yields were statistically analyzed by ANOVA (Statistica) to evaluate the influence of increasing total soil Cu and soil amendment on the mobility, availability and phytoavailability of Cu, Cr, Zn, and As. All analytical determinations were performed in two replicates. Differences were considered significant if the p-value was $p < 0.05$. R2 was the determination coefficient of the linear regression curve.

3. Results and discussion

3.1. Soil and soil pore water characteristics

Total soil As, Cr, and Zn were in the common ranges for French sandy soils but total soil Cu exceeded its background level and threshold value for soil contamination and risk assessment, *i.e.* 35 mg Cu kg^{-1} (Baize, 1997; Baize et al., 2002), for such coarse sandy soils (Tab. 1). For soils sampled in the field plots, total soil content (in mg kg^{-1}) varied between 5.3–6.9 for As, 16.2–19.4 for Cr, 35–74 for Zn and 163-1170 for Cu (Tab. 1). Total soil As, Cr, and Zn did not differ very much between the plots. Globally, Cu was the main contaminant of the topsoils. The physico-chemical characteristics (pH, DOC, EC, cations and anions) of the SPW, and the TOC of the soil samples are presented in Tab. 2.

Table 1: Total As, Cu, Cr and Zn concentrations in the topsoils (2010) adapted from Kolbas et al. (2011)

mg kg^{-1}				
plot#	Cu	Zn	Cr	As
T163	**163**	**73.8**	17.7	6.54
T268	**268**	**58.1**	16.4	5.33
T382	**382**	**50.1**	17.4	5.61
T518	**518**	**50.7**	19.4	6.90
T753	**753**	39.0	16.2	5.35
T832 (unt)	**832**	35.2	18.8	5.73
T1170	**1170**	**59.8**	16.3	6.19
Control soil[a]	**21**	**51**	18	3.60
BL[b]	3.2–8.4	17–48	14.1–40.2	1–25

[a]Control soil (Mench and Bes 2009).
[b]BL: background levels (Baize 1997; Baize and Tercé 2002). Bold letters indicate concentrations exceeding (>10%) background levels in French sandy soils.

The SPW pH decreased slightly from 2010 to 2011 for all soils (Tab. 2). The pH of the untreated soil was lower than that of the amended soils in 2010 and 2011, still reflecting the dolomitic limestone and compost addition in these soils in 2008. The control soil was slightly more alkaline than both the amended and untreated soils. The DOC and TOC values were little changes between 2010 and 2011. However, the DOC and TOC values peaked in 2011 for the CTRL soil compared with other soils and values for the CTRL soil in 2010. The Unt soil had the lowest TOC values. The EC values in 2011 were higher than in 2010 for all samples except the T163 and T268 soils. Our results agreed with previous findings for this field trial (Kolbas et al., 2011), Singh et al. (2007) also investigated the effect of organic amendment on the aided phytoextraction and reported highest pH and TOC values in the amended soils.

Table 2: Chemical characteristics of the SPW and soils (2010 and 2011).

Plot	TOC (%)	pH	EC (µS cm^{-1})	DOC (mg L^{-1})
2010				
T163	0.89±0.10	7.57±0.18	1923±210	66.24±0.01
T268	1.04±0.05	7.67±0.20	1111±190	47.66±0.12
T382	0.86±0.07	7.65±0.22	1076±122	81.3±0.13
T518	1.01±0.08	7.79±0.41	1404±132	47.64±0.16
T753	1.2±0.06	7.66±0.44	1006±111	45.01±0.02
T832 (unt)	0.59±0.04	6.48±0.19	839±98	46.35±0.04
T1170	1.07±0.05	7.74±0.21	1021±107	104.1±0.05
CTRL	0.63±0.09	7.95±0.18	574±65	63.06±0.09
2011				
T163	1.18±0.01	7.17±0.13	1423±176	67.86±0.07
T268	1±0.01	7.27±0.17	1011±123	55.23±0.35
T382	1.01±0.00	7.25±0.26	1676±201	39.51±0.12
T518	1±0.01	7.39±0.22	1504±232	46.2±0.10
T753	0.96±0.01	7.26±0.19	1206±182	48.45±0.08
T832 (unt)	0.72±0.01	5.88±0.24	1339±188	35.37±0.10
T1170	1.2±0.00	7.34±0.32	1321±123	34.65±0.04
CTRL	3.94±0.18	7.77±0.15	1074±198	124.2±0.64

3.2 Mobility of Cu, Cr, Zn and As

Concentrations of PTTE in SPW are relevant indicators of plant exposure to metals such as Cu for phytoextraction studies (Sauvé, 2003; Tandy et al., 2006; Forsberg et al., 2009). SPW samples from all tested soils showed a clear difference in the total dissolved concentration of PTTE between 2010 and 2011. The crop rotation on the tested plots, as well as annual leaching (Marchand et al. 2011), reduced the average concentrations of Cu, Zn, As, and Cr of all the treatments significantly from 2010 to 2011 by nearly 61%, 58%, 60% and 40% respectively (Fig. 2). For our field plots, Cu removal by shoots, capitula and seeds of sunflower in year 1 roughly reached 20-116 g Cu ha^{-1} depending on soil Cu contamination and sunflower genotype (Kolbas et al., 2011). In year 2, Cu removal by the aboveground parts of sunflower was roughly 20 g Cu ha^{-1}in the CTRL plot and increased from 16 (T268 soil) to 141 (T753 soil) g Cu ha^{-1} in the contaminated plots (Mench, unpublished data). In year 3, shoot removals by aboveground parts of tobacco were 27 g (CTRL soil), 58 g (untreated soil) and 145-183 g (amended soils) Cu ha^{-1} (Kolbas, 2012).

The total dissolved Cu concentrations in the SPW for the contaminated soils in 2010 and 2011 were higher than values for Zn, As, and Cr (Fig. 2) and the CTRL soil, i.e. 203.12 µg Cu L^{-1} in 2010 and 115.13 µg Cu L^{-1} in 2011 (Fig. 2.a). Total dissolved Cu concentrations in the SPW increased in 2010 and 2011 linearly with total soil Cu ($R^2 > 0.9$) confirming previous findings (Hattab et al., 2015). This agreed with Gonzalez et al. (2014), Kolbas et al. (2011) and Salati et al. (2010) who tested the efficiency of phytoextraction to reduce Cu excess in the soil pore water.

Total dissolved Zn concentrations in the SPW of the untreated and the control soil in 2011 were higher than those in the amended soils especially in 2011 (Fig. 2.b). Total dissolved Zn concentration in the SPW was correlated with the pH (R^2= 0.77), DOC (R^2> 0.53) and the TOC (R^2> 0.59) of soil, 0.59 respectively) in 2011 but did not show any correlation with other soil and SPW parameters. Our results agreed with Hattab et al. (2014) who tested the effect of compost (OM) and dolomitic limestone (DL), singly and in combination, on the SPW Zn concentration of Zn. They found that Zn mobility was reduced in the amended plots as compared to the untreated and control soils.

Total dissolved Cr and As concentrations in the SPW of all tested soils significantly decreased between 2010 and 2011, except for Cr in the Unt soil and the T1170 soil (Fig. 2 c and d). Total dissolved Cr and As concentrations in the SPW in 2011 were lower ($p<0.0001$) in contaminated soils compared with the control soil.

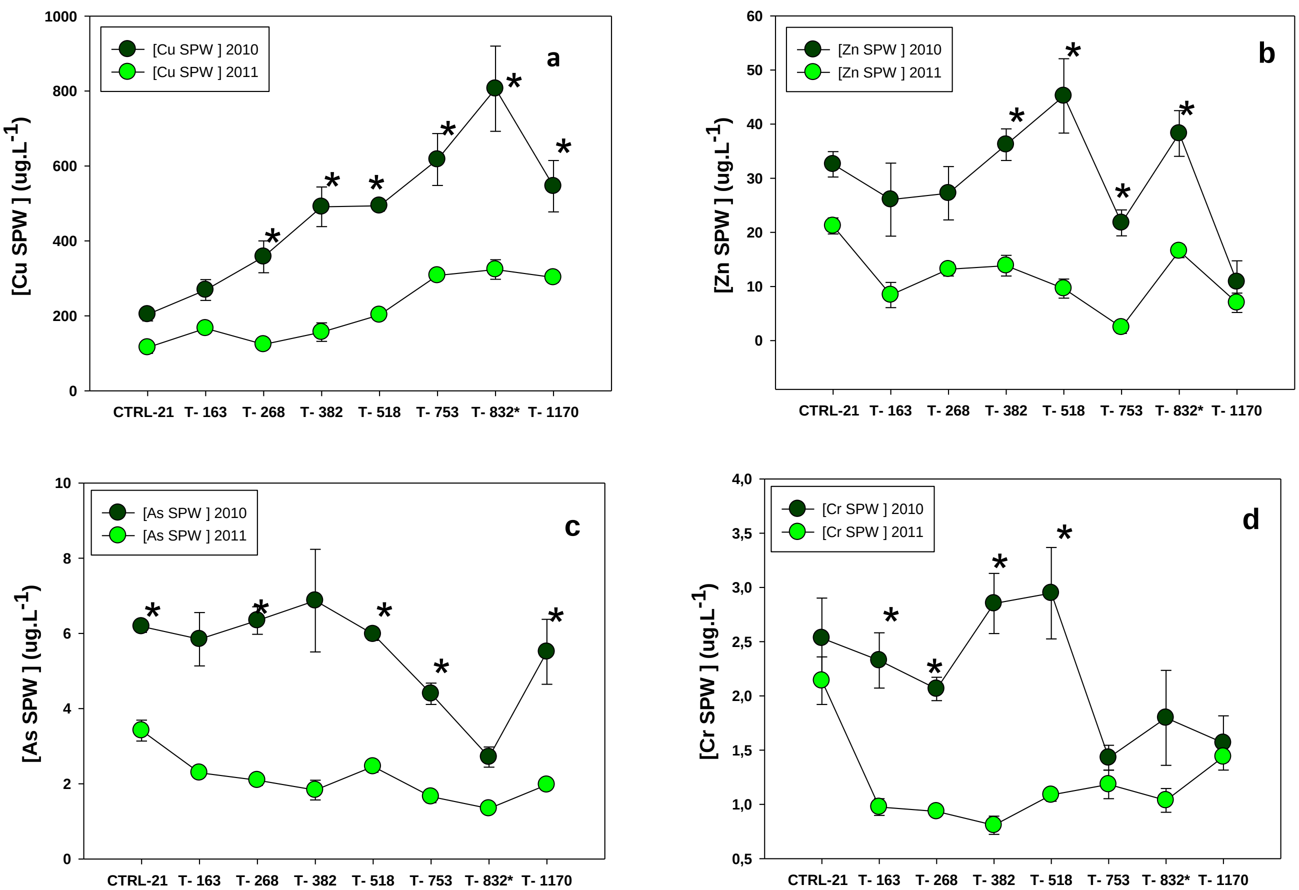


Figure 2: SPW concentrations of Cu, Zn, As and Cr measured in 2010 and 2011. Values are mean ± standard error (n=3). * indicate a significant difference ($p<0.05$).

3.3. Available soil Cu and Zn fractions

Data were obtained for available soil Cu and Zn fractions. Available Cu concentration in the contaminated soils varied from 116.123 to 11.25 µg L^{-1} in 2010 and from 11.75 to 40.02 in 2011 (Fig. 3). This available fraction was generally low compared to total soil Cu, i.e. <0.01 % in 2010 and <0.007 % in 2011. This available soil Cu fraction increased with total soil Cu, except for the CTRL soil in 2010. For three out of eight soil samples, i.e. CTRL, T832 and T1170 soils, the available soil Cu fraction decreased in 2011 compared to 2010, but no clear influence of the crop rotation was marked on the whole soil series. The CTRL soil had lowest total soil Cu and both TOC and DOC increased in 2011 (Table 2) which may promote the Cu complexation. The T832 and T1170 soils had the highest total soil Cu but their soil pH decreased in 2011which did not explain the decreased available soil Cu fraction. Available soil Cu, total soil Cu, total dissolved Cu concentration in the SPW and the SPW pH were correlated in 2011 ($R^2 > 0.6$). In contrast, no correlation was found between the soil and SPW parameters and the available soil Cu in 2010.

Available Zn concentrations ranged from 11.28 to 19.42 µg L^{-1} in 2010 and from 1.29 to 12.26 in 2011 in the contaminated soils. They declined from 2010 to 2011, except in the T1170 soil. The available soil Zn represented a low fraction compared to total soil Zn, i.e. <0.1% in 2010 and <0.04 % in 2011.

Available Zn and Cu concentrations in the soils were investigated in field plots managed by aided phytostabilisation at the same site 4 years after the incorporation of compost and dolomitic limestone, singly and in combination (OMDL) by Hattab et al. (2014) and similar results were found. The decrease in the available concentration of Cu can be explained by the formation of Cu–SOM complexes, particularly with non-soluble, high molecular mass organic acids which can decrease Cu phytoavailability (Balasoiu et al., 2001; Bolan and Duraisamy, 2003). The decrease in Zn availability in a contaminated soil can be explained by the presance of the organic and inorganic amendments, which can increase the precipitation and sorption of available Zn on the mineral phases (Lee et al., 2009).

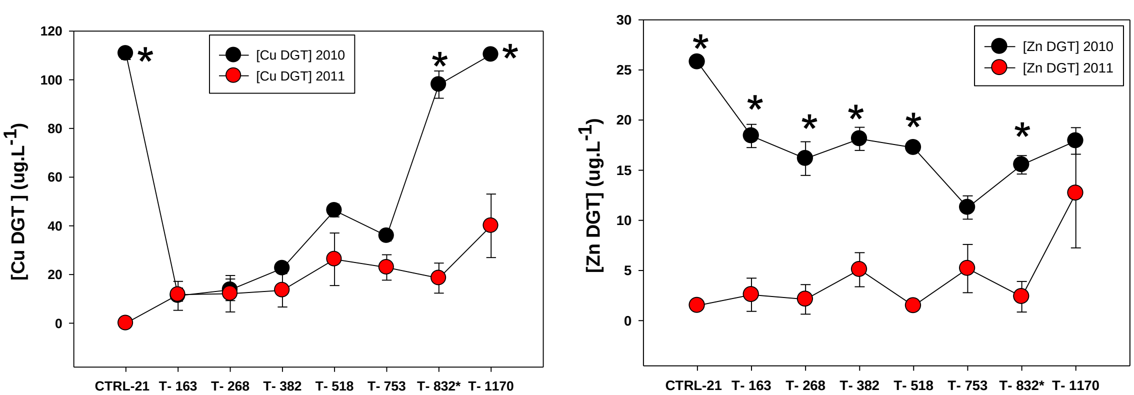


Figure 3: Available Cu and Zn concentratuion in the soil samples measured by DGT in 2010 and 2011. Values are mean ± standard error (n=3). * indicate a significant difference (p<0.05).

3.4. Phytoavailability of Cu, Cr, Zn and As

3.4.1. Concentration of PTTE in the bean primary leaves

Generally the Cu, Cr, Zn and As concentrations in the primary leaves of beans (BL) were higher in 2010 than in 2011, whereas the BL biomasses did not display general changes (Tab. 3). In average, these decreases were roughly 49% for Cu, 43% for Cr, 70% for Zn and 22% for As. Our results agree with Zhou et al. (2015) reporting that the repeated phytoextraction by *Sedum plumbizincicola* decreased the trace element phytoavailability in the soil especially for Zn.

Increase in total soil Cu enhanced the foliar Cu concentrations (Tab. 3). Data splitting according to two clusters for total soil Cu values (i.e. 200-500 and 800–1200 mg Cu kg^{-1}) resulted in a linear relationship with the foliar Cu concentration in 2010 and 2011, such concentrations increased from 40.6±1.3 µg Cu L^{-1} to 151.1±1.6 µg Cu L^{-1} (UNT soil) in 2010 and from 22.8±0.3 µg Cu L^{-1} to 65.5 ±0.4 µg Cu L^{-1} in 2011.

The highest foliar Cu concentrations in both 2010 and 2011 occurred in the untreated soil, showing likely the remaining beneficial effects of the initial soil amendment and enhanced crop yields. Influence of soil amendments for reducing both soil Cu exposure and plant Cu concentration was previously reported (Garrido et al., 2005). Hattab et al. (2014) investigated field plots managed by aided phytostabilisation at this site. They also found that beans grown on the untreated soil had higher foliar Cu concentrations than beans cultivated on the amended plots. Phytotoxic ranges of Cu for most plants are (in mg Cu kg^{-1}), e.g., 15–30 (MacNicol and Beckett, 1985), 25–40 (Chaney 1989), and 10–70 (Gupta and Gupta 1998). Accordingly the foliar Cu concentrations of our plants exceed these upper critical threshold values, especially for beans from in the untreated soil which had the highest total soil Cu. The foliar Cu concentrations were correlated with total soil Cu (R^2=0.96 in 2010 and 0.99 in 2011), total Cu concentration in the SPW (R^2 = 0.86 in 2010 and 0.94 in 2011) and the available soil Cu fraction (R^2=0.53 in 2010 and 0.58 in 2011) confirming previous findings with sunflower (Kolbas et al., 2011). Foliar Zn concentration in 2011 negatively correlated with total soil Cu (R^2= -0.88).Foliar Cr and Zn concentrations correlated with DOC and TOC in 2011 (R^2> 0.65). Correlations were weak between the foliar Cr, As and Zn concentrations measured in 2010 and 2011 and the other soil and plant parameters measured.

Table 3: Dry weight biomass (g DW plant^{-1}) and concentrations of Cu, Cr, Zn, and As (mg kg^{-1}) of the bean primary leaves.

	Dry weight (g) 2010	Dry weight g (2011)	[Cu] (mg Kg^{-1}) 2010	[Cu] (mg Kg^{-1}) 2011	[Zn] (mg Kg^{-1}) 2010	[Zn] (mg Kg^{-1}) 2011	[Cr] (mg.Kg^{-1}) 2010	[Cr] (mg Kg^{-1}) 2011	[As] (mg Kg^{-1}) 2010	[As] (mg Kg^{-1}) 2011
T163	0.12 ±0.00 ***	0.11±0.01 NS	40.6±1.3 ***	22.8±0.3 ***	72.0±0.5 ***	46.0±0.5 ***	2.83±0.13 ***	0.71±0.12 ***	0.42±0.01 ***	0.20±0.03 NS
T268	0.05±0.00 ***	0.10±0.00 NS	74.9±0.4 ***	29.0±0.1 ***	77.8±0.9 ***	49.3±0.3 ***	2.60±0.16 ***	1.00±0.01 ***	1.33±0.04 ***	0.16±0.01 NS
T382	0.21±0.01 ***	0.12±0.01 NS	60.1±0.01 ***	29.9±0.4 ***	56.2±0.8 ***	39.3±0.6 ***	2.17±0.09 ***	0.89±0.09 ***	0.70±0.00 ***	0.19±0.02 NS
T518	0.15±0.00 ***	0.11±0.01 NS	70.4±0.4 ***	40.3±0.2 ***	46.3±0.5 ***	38.8±0.3 ***	1.63±0.03 ***	1.20±0.08 ***	0.81±0.01 ***	0.25±0.01 NS
T753	0.09±0.00 ***	0.11±0.02 NS	142.5±2.1 ***	59.9±0.2 ***	68.9±1.2 ***	39.8±0.5 ***	1.29±0.03 ***	0.64±0.02 ***	1.17±0.06 ***	0.20±0.06 NS
T832 (unt)	0.12±0.01 ***	0.13±0.00 NS	173.1±1.6 ***	86.1±0.3 ***	40.4±0.4 ***	33.3±0.5 ***	1.47±0.05 ***	0.67±0.04 ***	1.34±0.06 ***	0.18±0.01 NS
T1170	0.09±0.00 ***	0.10±0.00 NS	151.1±1.6 ***	65.5±0.4 ***	67.7±1.5 ***	39.1±0.2 ***	3.75±0.09 ***	0.95±0.05 ***	1.13±0.05 ***	0.15±0.01 NS
CTRL	0.09±0.00 ***	0.10±0.00 NS	38.0±1.2 ***	19.5±0.3 ***	83.07±0.56 ***	53.65±0.07 ***	3.39±0.25 ***	1.46±0.09 ***	1.60±0.03 ***	0.23±0.02 NS

Effect of blocks: Significance level: NS : Not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

3.4.2 Mineral masses of Cu, Cr, Zn and As in the bean primary leaves

The mineral masses of Cu, Cr, As, and Zn in the primary leaves of beans cultivated in potted soils (mg/pot) was computed with foliar element concentrations (µg g^{-1} DW) and the primary leaf biomass (g DW pot^{-1}) (Fig. 4). The mineral masses of Cu, Cr, As, and Zn decreased significantly from 2010 to 2011, especially for Cu. In average, the mineral masses decreased by 49% for Cu, 55% for Cr, 78% for As, and only 34% for Zn.

Globally, the Cu mineral masses of bean primary leaves (Cu_{mm}) increased with total soil Cu, but peaked in the untreated soil due to its high Cu contamination and lower soil pH leading to a high foliar Cu concentration. Lower foliar Cu concentration and primary leaf DW yield explained the lower Cu_{mm} of beans on the T1170 soil compared to the untreated soil (T832). Total soil Cu and total Cu concentration in the SPW in 2010 and 2011 were correlated ($R^2 > 0.9$) with Cu_{mm}.

The Zn mineral masses of bean primary leaves (Zn_{mm}) were mainly driven by the primary leaf DW yield (Fig. 4 and Tab. 3), explaining its peak for the T382 beans in 2010. Except for this last case, Zn_{mm} globally decreased as Cu_{mm} raised. The Cr mineral masses of bean primary leaves (Cr_{mm}) were lower than Cu_{mm} and Zn_{mm}. Two lower Cr_{mm} values in 2010 corresponded to low primary leaf DW yield. The As mineral masses of bean primary leaves (As_{mm}) were the lowest compared to Cu_{mm}, Zn_{mm}, and Cr_{mm}. The Cu_{mm} did not influence Cr_{mm} and As_{mm}. Lower Cr_{mm} and As_{mm} values in 2011 in overall reflected the lower SPW Cr and As concentrations in 2011 (Fig. 2). The Cr_{mm} showed a good correlation with total soil Cr and total Cr concentration in the SPW in 2011 ($R^2 > 0.5$). In contrast a negative correlation was found between As_{mm} in 2010 and the total soil As ($R^2 = -57$).

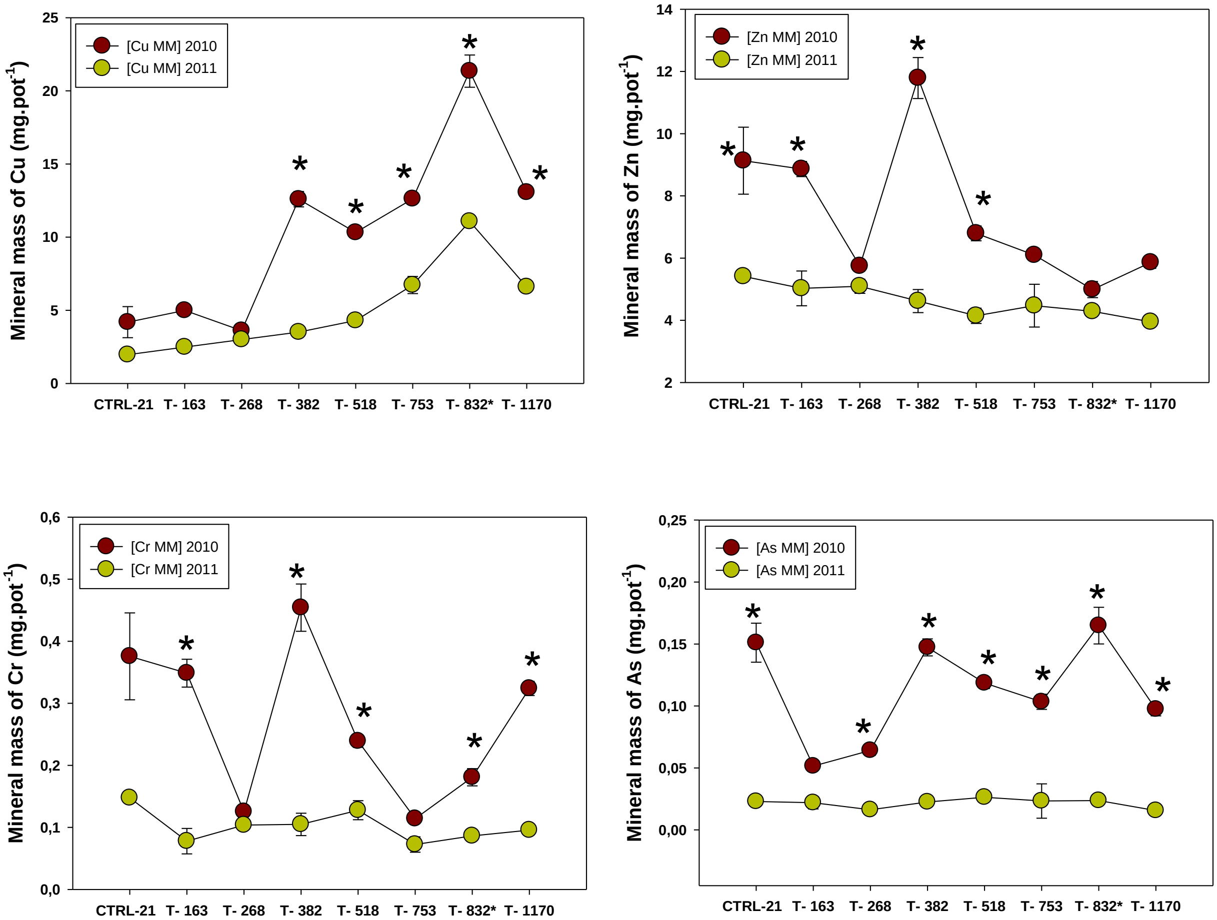


Figure 4: Mineral mass of Cu, Zn, Cr and As measured in the bean leaves. Values are mean ± standard error (n=4). * indicate a significant difference ($p<0.05$).

4. Conclusion

Changes in the mobility (using soil pore water), availability (using DGT) and phytoavailability of Cu, Zn, Cr and As (using plant testings with beans) were investigated at a wood preservation site, in topsoils of field plots, either amended or not with a single addition of dolomitic limestone (DL) and compost (OM), phytomanaged with a crop rotation of sunflower and tobacco, after two and three years. For purpose of comparison a similar uncontaminated control plot was also investigated. Total dissolved Cu concentrations in the SPW were higher than for Zn, As, and Cr. Total dissolved concentrations of Cu, Zn , Cr and As in the SPW decreased in year 3 as compared to year 2, likely due to shoot removals by the crop rotation and the lixiviation. Available soil Cu and Zn fractions also declined in year 3. For the contaminated field plots, available soil Cu fraction increased with the total soil Cu soil, but depended also on soil pH. The phytoavailability of Cu, assessed by the foliar Cu concentration and the Cu mineral mass of the primary leaves of beans, was reduced in year 3.

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